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## AN OCCURENCE OF ALBINO EGGS OF THE SPOTTED SALAMANDER, *AMBLYSTOMA PUNCTATUM* L.

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The folowing account could not have been written at all except that Dr. C. C. Mercer kindly placed at my disposal the facilities of the Medical College, and that Prof. C. W. Hargitt gave me access to literature on the subject.

About the middle of last March, while searching for amblystoma and frog eggs, several small masses of white eggs were found. As they were supposed to be unfertilized and already decomposing, only one mass was brought to the laboratory, where an examination under the microscope showed the eggs to be developing regularly, the surface presenting a network of cells normal in all respects except that there were no pigment granules present. A day or two later the remainder of the eggs were obtained (about 100 in all), and various stages in their development have been preserved for study.

All of the growth phenomena proceeded apparently regularly, the egg lengthening, neural folds forming and closing, the gill ridges and tail appearing and finally life being manifested by the twitching of the body when the jelly mass was disturbed. Just at this stage there began to be noticable to the naked eye a slight grayish mottling of the sides of the body. This pigmentation increased as the tadpoles grew and when they had reached a length of about eighteen mm. they could not be distinguished, by the naked eye, from normal embryos raised in dim light in the laboratory, but were a little lighter in color than embryos freely exposed to the sunlight.

An examination of the preserved material shows that in none of the stages are there present any brownish black pigment granules. Embryos about six or seven mm. long show, just along and above the midline of the side of the body, many small, angular, dark spots, quite uniformly scattered along a band about one-quarter as wide as the dorso-ventral diameter

of the body. In an embryo ten mm. long the spots have increased considerably in number, spreading both dorsally and ventrally, and have sent out numerous branches which in many instances anastomose freely. One mass of such spots is seen just in front of the gills, another just behind the gills, while those along the side of the body dorsal to the midline are mainly disposed in four groups. Those ventral to the midline are more uniformly distributed. A few spots are present on the gills and a few are found in the tail region of the dorsal fin. In an embryo thirteen mm. long the branches anastomose much more freely, and the head, before nearly spotless, has become thickly covered. At fifteen mm. the spots are fusing together in numerous instances.

The surface of normal eggs is black or dark brown in color, and as the embryo grows it continues quite uniformly dark brown (except ventrally) for some time, becoming finally in embryos twenty or more millimeters long a grayish black color. In a normal embryo nine mm. long this uniform brownish color is seen to be augmented along the sides by darker patches composed of the black branching pigment spots, which in an embryo twelve mm. long have spread quite freely over the surface.

Pigment in the salamander larva has been recognized as occurring in three ways: first, as minute brownish-black spherules in the epithelial cells; second, in branching pigment cells with processes passing between the epithelial cells; and third, in the ramified pigment cells of the cutis. In this paper no distinction is made between the second and third classes.

The surface pigmentation of the normal egg is of the first class and a section of such an egg when the epiblast is several cells deep, shows the outer layers of cells to contain large quantities of such granules, chiefly massed near the free surface of the cells, but extending also inward, mainly along the cell wall. Similar pigmented granules are distributed less abundantly over the free surface of the cells lining the mesenteron, and also to some extent are present in some of the yolk cells lining the blastocoel.

Sections of a normal tadpole thirteen mm. long show the surface epithelium to be granularly pigmented as above, but the granules are more compactly arranged in a layer close to the surface. Just beneath the epidermis the branching pigment cells are numerous, the branches extending usually parallel or obliquely to the surface; at places in the tail they appear to form almost a continuous layer. In the gills the branches ramify more freely among the surrounding cells. The ear and nostril invaginations are quite deeply pigmented by the brownish black granules; the pigmented layer of the dorsal portion of the retina is composed mainly of the subepidermal branching cells, while the ventral portion seems to derive its color from the pigmented granules. The cells of the nervous system contain numerous rounded black pigment bodies, which are present to a lesser extent throughout the mesodermal cells of the body.

In a tadpole eight mm. long, the subepidermal, branching pigment cells are not so abundant nor so fully developed as in the larger individual. There are rounded pigment bodies in the mesoderm, but not in the nervous system, which is colored brownish by the pigment granules. The pigment of the retina of the eye seems to be of the epidermal granular nature, rather than of the subepidermal cellular character.

Sections of an albino embryo with closed nervous system show no traces of pigmentation whatever, either granular or cellular. Sections of an embryo six mm. long show subepidermal pigment cells appearing, but there are as yet no traces of pigmentation in the eye or ear invaginations.

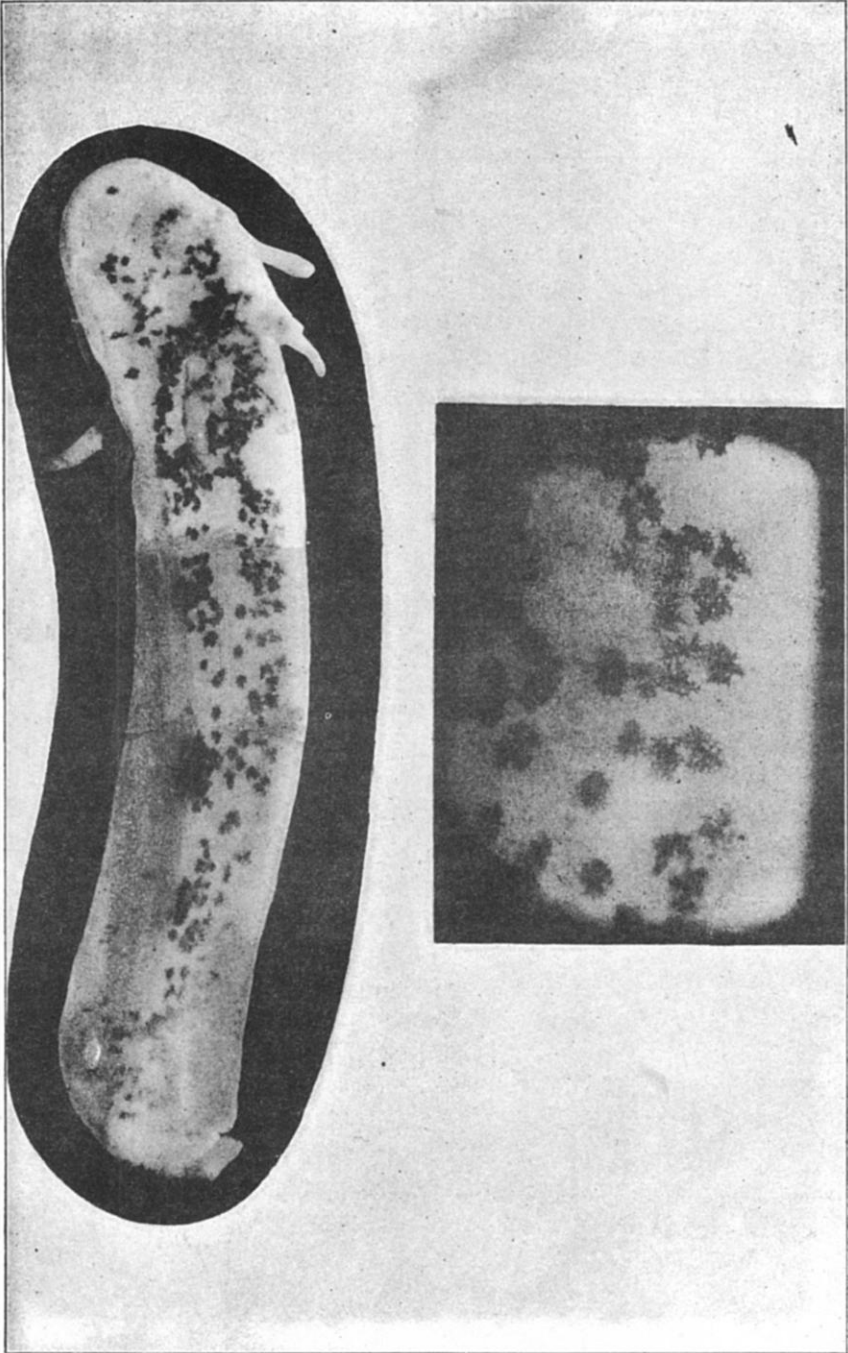
In a tadpole ten mm. long the subepidermal pigment cells are much more numerous than in the previous stage and are branching out in all directions more freely than those in the slightly older stage of normal embryos. The ear invaginations are entirely devoid of pigment but the retina is being pigmented dorsally and downward along the back of the optic cup, probably by the branching pigment cells. The lower side of the retina is not yet pigmented, and in the adjacent subepidermal tissue there are only two or three pigment cells in the

neighborhood of each eye. The cells of the nervous system and also those of the mesoderm throughout the body are without any traces of the rounded pigment bodies so numerous in the normal embryo.

A SHORT LIST OF PAPERS BEARING MOST DIRECTLY UPON THE SUBJECT

- CAMARANO, LOR.: Di alcuni girini albini e delle cause dell' albinismo. Boll. Musei Zool. Anat. Comp. Torini. T. 4, No. 64.
- FISCHER-SIGWART, H.: Sur l'albinisme chez les larves de *Rana temporaria*, avec quelques remarques sur l'albinisme en generale. Verhandlgn. Schweiz. naturf. Ges. Soloth, 1888, p. 59.
- LESSONA, MICH.: Dello albinismo nei girini della *Rana temporaria* L.: Atti. R. Accad. Sc., Torino. Vol. 16, Disp. 1, p. 94.
- FISCHER, A.: Über Beeinflussung and Entwicklung des Pigments. Arch. f. mikr. Anat. Bd. 47, Hft. 4, p. 719.
- FISCHEL, A.: Pigmentation of *Salamandra maculata*. (Abstr. in Jour. Roy. Micr. Soc. 1896, p. 611.)
- WINKLER, F.: Origin of Pigment in *Bufo*. Mitth. Embryol. Inst. K. K. Univ. Wien, 1892, p. 64. (Abstr. in Jour. Royal Micr. Soc. 1896).

PLATE VI



**PLATE VI.**

Fig. 1. Photomicrographs of *Amblystoma punctatum* 10 mm. long, matched to show distribution of branching pigment cells.

Fig. 2. Portion A-B of Fig. 1, more highly magnified to show character of branching pigment cells.